Evaluation of Efficacy of Different Denture Disinfectants on Biofilms Formed on Acrylic Resin

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Abstract

Purpose Health professionals, especially in dentistry, are involved at high risk of microbial cross contamination. There are many studies that reported the risk of cross-contamination in prosthetic laboratories. Although much importance has been given to infection control practices, such as the barrier technique, sterilization, and disinfection of the dental office and instruments, less emphasis has been given for the disinfection of dentures. The dentures in mouth are prosthetic medical devices that create an appropriate habitat for both pathogenic and nonpathogenic organisms to nurture. To avoid cross-contamination, denture disinfection is mandatory. This study evaluated the efficacy of four denture disinfectants on four most common organisms found in denture biofilm at two different time intervals.

Materials and Methods Three hundred twenty denture base acrylic resin specimens were fabricated by means of wax patterns with dimensions diameter 15 mm × 4 mm. The contamination of samples was done by the four microorganisms taken in study that were *Staphylococcus aureus*, *Candida albicans*, *Pseudomonas aeruginosa*, and *Escherichia coli*. The contaminated samples were disinfected by immersion procedure in four disinfectants that were sodium hypochlorite, glutaraldehyde, peracetic acid, and chloroxylonol.

Results Sodium hypochlorite was the best disinfectant resulting in complete elimination of microorganisms in 5 minutes immersion time. Glutaraldehyde and chloroxylonol were also effective against microorganisms. Minimum 10 minutes immersion time was required to completely eliminate the microorganisms. Peracetic acid was least effective of all showing the least reduction in microbial growth. Among the organisms, *S. aureus* showed the maximum resistance to disinfection, and *C. albicans* was the least resistant.

Keywords

- disinfectants
- infection control
- microorganisms
- acrylic resin

Introduction

Invisibility does not mean nonexistence; opportunity makes the presence felt. It is from the vitality of the atmospheric particles that all the mischief arises.

Nowadays there is high consternation regarding the spread of infectious diseases. More awareness is on several ways of cross-contamination external to the dental operator, facilitated by the dentist and the dental auxiliary personnel. Patients are becoming more sophisticated in their scrutinizing of the dental and medical professional's approach to asepsis.

Health professionals, especially in dentistry, are also involved at high risk of microbial cross-contamination. There are many studies that reported the risk of cross-contamination in prosthetic laboratories.¹ Although much importance has been given to infection control practices, such as the barrier technique, sterilization, and disinfection of the dental office and instruments, less emphasis has been given for the disinfection of dentures. The dentures in mouth are prosthetic medical devices that create an appropriate habitat for both pathogenic and nonpathogenic organisms to nurture.² So, these dental prostheses create a medium for the transfer of organisms from patients to the dental and laboratory personnel. To avoid cross-contamination and improve the denture hygiene, denture disinfection is mandatory. The use of various disinfectants has been reported. The literature differs markedly regarding the concentration and duration of disinfectants used. Henceforth, this study was intended to evaluate the efficacy of four denture disinfectants on four most common organisms found in denture biofilm at two different time intervals.

Materials and Methods

Specimen Fabrication

Three hundred twenty denture base acrylic resin specimens were fabricated by means of wax patterns with the dimensions (diameter 15 mm, width 4 mm). The wax samples were prepared in the custom-made metal mold. The wax discs were then invested in dental flasks with type III gypsum product, i.e., dental stone, and dewaxing was done. The heat cure acrylic resin was prepared with polymer:monomer ratio of 3:1 by volume in a mixing jar by using the measuring cylinder. The stone mold was packed in dough stage. Polymerization of acrylic resin was done following a short curing cycle (74°C for 2 h followed by terminal boiling for 1 hour) in acrylizer. After curing, overnight bench cooling was done. The cured samples were deflasked carefully, and finishing was done.

All the samples were sterilized by microwave sterilization. Microwave oven irradiation for sterilization of non-autoclavable dental instruments and dentures was first introduced by Rohrer and Bular. Microwave radiations have thermal effect and non-thermal effect. The thermal effect is due to changes on cell structure, alteration of cell membrane permeability, and cell death. The heat generated by microwaves upon the organic matter is assumed to be the causative factor for these modifications. The non-thermal effect relates to the destruction of microorganisms at temperatures lower than the thermal destruction point. The probable justification lies on the selectivity of microwave irradiation absorbance by certain biochemical molecules, such as protein, nucleic acid, and the protein-lipopolysaccharide compounds of cell membranes, for example, the interaction between electromagnetic field produced by the microwaves, and the cell molecules would lead to cell destruction.

Samples were placed in microwave safe container containing sterile water. Sterilization was done for 3 minutes at 650 W.

Contamination of the Specimens

The contamination of samples was done by the four microorganisms taken in study. Sixty samples from each group (15 from each subgroup) were contaminated. First, each microbial strain was cultured in a culture medium: Tryptic Soy Broth. Then, each acrylic resin specimen was transferred to containers containing culture medium broth (200.0 mL) with 1.0% microbial inoculum at 0.5 McFarland scale. These containers were employed for the formation of biofilm of microorganisms onto the sample's surfaces. The containers with the samples and inoculums had been placed in the incubator for 48 hours at 37°C. After incubation, there was growth of the particular organism over the samples. The contaminated samples were removed from the broth, gently washed in water, and were employed for disinfection.

Disinfecting the Samples

Samples from the broth were disinfected by immersion procedure using different disinfectants (sodium hypochlorite, glutaraldehyde, peracetic acid, and chloroxylenol). Ten samples from each subgroup were immersed in disinfectant. Five samples were immersed for 5 minutes and five samples for 10 minutes. The five samples in each subgroup, which were contaminated, were not dipped in disinfectant (positive control). After removing from disinfectant, again all the samples were washed and cultured for colony formation.

Microbial Counting

The samples were placed in contact with appropriate culture medium contained in a Petri plate. Samples were discarded, and the Petri plates were incubated at 37°C for 48 hours. After incubation, the colony-forming unit (CFU) for each culture plate was counted with colony counter (►Figs.1–4). The results obtained were compared to evaluate the efficacy of different denture disinfectants at two time intervals.

Fig. 1 Blood agar plates showing CFU for *Pseudomonas aeruginosa* after 5 and 10 minutes immersion. CFU, colony-forming unit.

Fig. 2 Blood agar plates showing CFU for *Candida albicans* after 5 and 10 minutes immersion. CFU, colony-forming unit.

Fig. 3 Blood agar plates showing CFU for *E. coli* after 5 and 10 minutes immersion. CFU, colony-forming unit.

Fig. 4 Blood agar plates showing CFU for *Staphylococcus aureus* after 5 and 10 minutes immersion. CFU, colony-forming unit.

Results

The results showing the efficacy of various disinfectants are shown in (► **Tables 1–4**). There is statistically significant difference among the final counts of microbes after the disinfection. Among the four disinfectants used in the study, sodium hypochlorite was the best disinfectant resulting in complete elimination of microorganisms in 5 minutes immersion time. Glutaraldehyde and chloroxylenol were also effective against microorganisms, but the complete disinfection could not be achieved in 5 minutes immersion. Minimum 10 minutes immersion time was required for glutaraldehyde and chloroxylenol to completely eliminate the microorganisms. Peracetic acid was the least effective of all showing the least reduction in microbial growth. Out of organisms *Staphylococcus aureus* showed the maximum resistance to disinfection, and *Candida albicans* was the least resistant.

Discussion

The human mouth is a repository for many pathogenic microorganisms that are the causative factors of various oral and systemic infections. These oral infections contribute in the pathological process of various systemic diseases, which affects the general prognosis of not only frail, elderly, and immunocompromised patients, but also of those who are healthy.³ In recent times, there is a consequent rise in the ratio of elderly within each population. This increase has led to increase in the proportion of population which are immunocompromised and/or have physical health problems such as stroke, aspiration pneumonia, cardiovascular disease, and diabetes. Amid these, aspiration pneumonia grounds for the maximum loss of life in the elderly. The pathogens of aspiration pneumonia, including *C. albicans* and *S. aureus*, show high potential to cause pneumonia in the elderly than in young adults.⁴ It has been documented that *C. albicans* strains have the capability to produce carcinogenic nitrosamines from saliva and foster neoplastic changes. The co-existence of *Pseudomonas* spp. and *C. albicans* in old population is a potential indicator of a high risk for pneumonia and heart disease.³ *Escherichia coli* is correlated to fecal contamination.

In dental laboratories, the prime source of cross-contamination is the prosthesis, especially the dentures of immune-compromised patients. During the routine laboratory procedures, such as polishing and trimming, the aerosols so produced contain the microorganisms adhered to these dentures which are capable of infecting the professional and objects used daily, such as polishing brushes, burs, lathe, and pumice stone.^{4,5}

In view of this, compliance with the universal biosecurity policies, such as the adoption of strict disinfection measures, is of paramount importance to prevent interpatient microbiological cross-contamination within the dental office and laboratory.⁶ Recommendations exist for the use of safety measures as well as for disinfection techniques. To address these cross-contamination concerns, the American Dental

Table 1 Results obtained for the antimicrobial effectiveness of sodium hypochlorite after 5 and 10 minutes immersion

Group			N	Mean	Standard deviation	Standard error
Sodium hypochlorite	<i>Staphylococcus aureus</i>	Positive control	5	213.8000	6.97854	3.12090
		5-minute dipping	5	1.2000	1.78885	0.80000
		10-minute dipping	5	0.2000	0.44721	0.20000
		Negative control	5	00	00	00
		Total	20	53.8000	94.83481	21.20571
	<i>Escherichia coli</i>	Control	5	227.2000	7.08520	3.16860
		5-minute dipping	5	0.4000	0.54772	0.24495
		10-minute dipping	5	0.0000	0.00000	0.00000
		Negative control	5	00	00	00
		Total	20	56.9000	100.92983	22.56860
	<i>Candida albicans</i>	Control	5	243.0000	13.37909	5.98331
		5-minute dipping	5	0.0000	0.00000	0.00000
		10-minute dipping	5	0.0000	0.00000	0.00000
		Negative control	5	00	00	00
		Total	20	60.7500	108.12998	24.17860
	<i>Pseudomonas aeruginosa</i>	Control	5	256.8000	5.35724	2.39583
		5-minute dipping	5	0.0000	0.00000	0.00000
		10-minute dipping	5	0.0000	0.00000	0.00000
		Negative control	5	00	00	00
		Total	20	64.2000	114.11287	25.51641

Association issued guidelines for disinfection in 1985, 1988, and 1996.⁷⁻⁹ Similar guidelines were given by the British Dental Association, Centre for Disease Control, Occupational Safety and Health Administration, and Environment Protective Agency. They all recommend use of disinfection for prevention of cross-contamination.

Disinfection of dentures should be done at a standard time interval to keep the dentures free from microorganisms. For disinfection, alternative methods have been proposed such as chemical disinfection,¹⁰ microwave,¹¹ and ultraviolet radiation.¹² However, chemical disinfection, preferably by immersion, seems to be the most reliable and practical method.¹³ Literature differs markedly in the concentration, type, and immersion times of disinfection protocols, making it difficult to assess the most appropriate method. Commonly used chemical disinfectants are alcohols, aldehydes, chlorine compounds, phenolics biguanides, iodine compounds, and quaternary ammonium compounds.¹⁴

Sodium hypochlorite and glutaraldehyde have been widely recommended for disinfection.¹⁵ Peracetic acid was introduced in market in 1998 and has been indicated for high-level disinfection and sterilization of critical and semi-critical articles.¹⁶ Chloroxylonol is being routinely used in household disinfection, but no study has been done on its use as denture disinfectant.

Therefore, the present study was conducted to evaluate the efficacy of above-mentioned four denture disinfectants on biofilms formed on acrylic resins at two different time intervals, i.e., 5 minutes and 10 minutes. A total 320 acrylic samples were prepared and were contaminated by four organisms *Staphylococcus aureus*, *E. coli*, *C. albicans*, and *P. aeruginosa*. Disinfection was done by immersion method for 5 minutes and 10 minutes in each disinfectant.

When the samples contaminated with all the four organisms were dipped in sodium hypochlorite, the results showed that sodium hypochlorite was highly effective against all the organisms. The results are in concurrence with the study

Table 2 Results obtained for the antimicrobial effectiveness of glutaraldehyde after 5 and 10 minutes immersion

Glutaraldehyde	<i>Staphylococcus aureus</i>	Control	5	213.4000	5.77062	2.58070
		5-minute dipping	5	28.0000	14.67992	6.56506
		10-minute dipping	5	0.8000	1.09545	0.48990
		Negative control	5	00	00	00
		Total	20	60.5500	91.56388	20.47431
	<i>Escherichia coli</i>	Control	5	222.0000	9.19239	4.11096
		5-minute dipping	5	18.8000	6.37966	2.85307
		10-minute dipping	5	0.2000	0.44721	0.20000
		Negative control	5	00	00	00
		Total	20	60.2500	96.26929	21.52647
	<i>Candida albicans</i>	Control	5	244.2000	5.31037	2.37487
		5-minute dipping	5	16.8000	6.79706	3.03974
		10-minute dipping	5	0.2000	0.44721	0.20000
		Negative control	5	00	00	00
		Total	20	65.3000	106.27576	23.76398
	<i>Pseudomonas aeruginosa</i>	Control	5	257.2000	10.08464	4.50999
		5-minute dipping	5	6.4000	3.64692	1.63095
		10-minute dipping	5	0.2000	0.44721	0.20000
		Negative control	5	00	00	00
		Total	20	65.9500	113.42444	25.36247

done by Bell et al¹⁷ who compared the biocidal effectiveness of 5.25% sodium hypochlorite (1:10 dilution) on acrylic strips inoculated with microorganism *S. aureus*, *E. coli*, and *C. albicans*. The result showed that sodium hypochlorite achieved complete disinfection of all the three microorganisms in 4 minutes. No significant difference was found in 5 minutes immersion and 10 minutes immersion for all the organisms suggesting that 5 minutes immersion in sodium hypochlorite is sufficient to achieve disinfection. This is in agreement with Rudd et al¹⁸ who studied the antimicrobial action of sodium hypochlorite for the sterilization of complete dentures and determined the time for which the prosthesis should be immersed to achieve this effect. The dentures were coated with the *S. aureus*, *Bacillus subtilis* (both vegetative and spore forms), *C. albicans*, *P. aeruginosa*, and *Streptococcus* (enterococcus) and then immersed in sodium hypochlorite for 1 and 3 minutes showed evidence of growth, whereas the dentures immersed for 5 minutes were sterile.

The results of immersion in glutaraldehyde showed that glutaraldehyde was effective in decreasing the microbial load

after 5 minutes, but 10 minutes immersion is required to achieve complete disinfection. The results are in accordance with the study done by Henderson et al¹⁹ who evaluated the disinfection of prosthesis with full strength Sporidicin (2% glutaraldehyde), 1:6 Sporidicin solutions, and 5.25% sodium hypochlorite (undiluted Clorox) and confirmed that after 10 minutes full strength, Sporidicin was effective in reducing or eliminating culturable aerobic bacteria. Bal et al²⁰ also concluded that 10 minutes immersion in 2% glutaraldehyde and 0.525% sodium was effective for disinfection and great reduction of microorganisms.

The results of peracetic acid immersion depicted that though the number of colonies were reduced, but complete disinfection was not achieved even after 10 minutes disinfection. The results are supported by the study of Stopiglia et al²¹ who concluded in their study that after 5 minutes disinfection with 0.2%, peracetic acid was effective against *C. albicans*, but was ineffective against *E. coli*, *S. aureus*, and *P. aeruginosa*.²² Ceretta et al²³ evaluated the effectiveness of peracetic acid in the sterilization of dental equipment and concluded that immersion of the materials in peracetic acid

Table 3 Results obtained for the antimicrobial effectiveness of chloroxylenol after 5 and 10 minutes immersion

Chloroxylenol	<i>Staphylococcus aureus</i>	Control	5	216.2000	4.71169	2.10713
		5-minute dipping	5	31.2000	5.58570	2.49800
		10-minute dipping	5	3.6000	1.67332	0.74833
		Negative control	5	00	00	00
		Total	20	62.7500	91.80005	20.52712
	<i>Escherichia coli</i>	Control	5	226.6000	11.54556	5.16333
		5-minute dipping	5	8.6000	1.94936	0.87178
		10-minute dipping	5	0.0000	0.00000	0.00000
		Negative control	5	00	00	00
		Total	20	58.8000	99.60638	22.27266
	<i>Candida albicans</i>	Control	5	250.4000	6.87750	3.07571
		5-minute dipping	5	6.8000	3.70135	1.65529
		10-minute dipping	5	0.0000	0.00000	0.00000
		Negative control	5	00	00	00
		Total	20	64.3000	110.33113	24.67079
	<i>Pseudomonas aeruginosa</i>	Control	5	263.6000	12.17785	5.44610
		5-minute dipping	5	22.4000	8.59069	3.84187
		10-minute dipping	5	0.8000	1.09545	0.48990
		Negative control	5	00	00	00
		Total	20	71.7000	114.25092	25.54728

solution at a concentration of 2,500 ppm for 20 minutes promoted complete sterilization. Sukhija et al concluded in their study that peracetic acid was most effective followed by glutaraldehyde and sodium hypochlorite; the latter disinfectants were comparable in their antimicrobial effect.²⁴

The results of immersion in chloroxylenol showed that chloroxylenol achieved disinfection after 10 minutes. Five minutes immersion was inappropriate. The literature reviewed did not show any study involving chloroxylenol as denture disinfectant. Chemically, chloroxylenol is a phenolic compound, and phenols have been recommended as denture disinfectants.²⁵

So, from results, it can be inferred that sodium hypochlorite was effective disinfectant for 5 minutes and 10 minutes against all the microorganisms taken in the study. Glutaraldehyde and chloroxylenol achieved complete disinfection after 10 minutes. For peracetic acid, disinfection could not be achieved even after 10 minutes. The results are in agreement with study of da Silva FC²⁶ in which they evaluated the efficacy of the effectiveness of various disinfectant solutions in the disinfection of acrylic resin specimens contaminated in

vitro by *C. albicans*, *Streptococcus mutans*, *S. aureus*, *E. coli*, or *B. subtilis*. The results showed that sodium hypochlorite showed the highest antimicrobial activity. Glutaraldehyde and chlorhexidine gluconate showed similar activity. Vinegar, sodium perborate, and sodium perborate-based tabs showed no sporicidal activity. The Council of Dental Therapeutics⁸ has also suggested the use of glutaraldehyde and sodium hypochlorite for 10 minutes for achieving complete disinfection.

Out of organisms, *S. aureus* was found to be the most resistant to disinfection and *C. albicans* to be the least resistant. It has been documented that some antiseptics and disinfectants are rather less effective to *S. aureus* strains that carry a plasmid carrying genes encoding resistance.²⁷ The study of Saha et al²⁸ showed similar results in which the effect of six types of antiseptics and disinfectants, namely Dettol (chloroxylenol), Savlon (chlorhexidine gluconate and cetrimide), iodine, phenyl, formalin, and hydrogen peroxide (H_2O_2) was studied on *S. aureus*, *Salmonella typhi*, *Shigella dysenteriae*, *Klebsiella* species, and *E. coli*. *S. aureus* showed the maximum resistance to disinfectants tested. *E. coli* and *P. aeruginosa* showed more resistance than *C. albicans*.

Table 4 Results obtained for the antimicrobial effectiveness of peracetic acid after 5 and 10 minutes immersion

Peracetic acid	<i>Staphylococcus aureus</i>	Control	5	213.2000	6.14003	2.74591
		5-minute dipping	5	79.6000	15.91540	7.11758
		10-minute dipping	5	42.4000	5.22494	2.33666
		Negative control	5	00	00	00
		Total	20	83.8000	82.32324	18.40804
	<i>Escherichia coli</i>	Control	5	219.0000	8.06226	3.60555
		5-minute dipping	5	25.2000	4.86826	2.17715
		10-minute dipping	5	1.6000	2.50998	1.12250
		Negative control	5	00	00	00
		Total	20	61.4500	93.99075	21.01697
	<i>Candida albicans</i>	Control	5	240.2000	8.28855	3.70675
		5-minute dipping	5	18.2000	6.05805	2.70924
		10-minute dipping	5	5.6000	1.14018	0.50990
		Negative control	5	00	00	00
		Total	20	66.0000	103.51710	23.14713
	<i>Pseudomonas aeruginosa</i>	Control	5	256.2000	7.22496	3.23110
		5-minute dipping	5	88.6000	8.23408	3.68239
		10-minute dipping	5	43.4000	5.94138	2.65707
		Negative control	5	00	00	00
		Total	20	97.0500	99.76471	22.30807

because generally the Gram-negative bacteria are more resistant to disinfection than yeast or fungi.²⁹

The present study recommends that sodium hypochlorite 5.25% (1:10 dilution), glutaraldehyde 2%, and chloroxylonol 0.48% for 10 minutes immersion time can be used in the dental clinic, in the laboratory, and for the patient to prevent cross-contamination and to maintain good denture hygiene for the patient.

Conclusion

To prevent the transmission of disease and for good oral hygiene of the oral cavity, denture disinfection is recommended. Immersion in 5.25% sodium hypochlorite (1:10 dilution) for 5 minutes and in glutaraldehyde and chloroxylonol for 10 minutes is highly effective in achieving complete disinfection. It has to be the responsibility of each associate of dental health team to make certain that all required measures are taken to fend off any cross-infection to both patients and to themselves.

Conflict of Interest

None.

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